Multi-locus sequence analysis of *Mycoplasma capricolum* subsp. *capripneumoniae* for the molecular epidemiology of contagious caprine pleuropneumonia

Lucía Manso-Silván, Virginie Dupuy & François Thiaucourt
CIRAD UMR 15, Montpellier, France
CCPP Reference Lab OIE FAO

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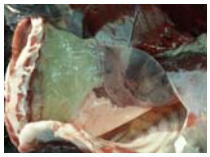


Introduction

Mycoplasma capricolum subsp. *capripneumoniae* (Mccp) is the agent of Contagious Caprine Pleuropneumonia (CCPP), a devastating disease included in the list of notifiable diseases of the OIE. Its exact distribution is not known but CCPP is prevalent in Africa and the Middle East. New outbreaks have recently been declared in Tajikistan and Mauritius and there is a risk of introduction in Europe from West Turkey. Furthermore, the isolation of Mccp from wildlife species in 2004 posed new questions regarding host specificity and the risk of CCPP introduction via importation of zoo animals. A fine molecular typing tool is required to address all the epidemiological questions that arise and to trace new epidemics. Molecular typing based on either 16S rDNA (1) or H2 locus (2) sequence analysis were limited by a reduced number of polymorphisms and 16S rDNA analysis did not show a good correlation with geographic origin. The objective of this study was to develop a Multi-Locus Sequence Analysis (MLSA) strategy such as that existing for *M. mycoides* subsp. *mycoides* SC (3).

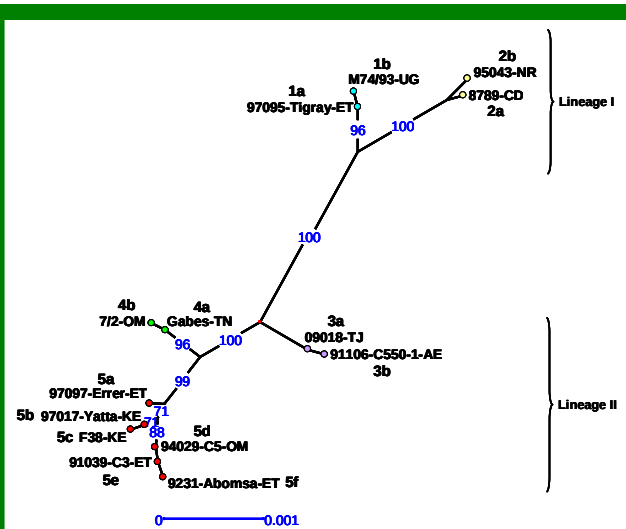

Fig. 2: Tree derived from distance analysis of 8 concatenated sequences (Unweighted Neighbor-Joining, nucleotide simple matching, pairwise gap block correction, Darwin 5.0). Bootstrap percentage values from 1000 resamplings are located at the nodes. A single strain representing each of the 14 sequence types is shown. See Table 1 for data on additional strains.

Results and Discussion

MLSA based on 7 loci resulted in higher number of polymorphisms and increased discriminatory power (39 variables providing 13 sequence types) as compared to H2 locus sequence analysis (12 polymorphisms, 8 sequence types). The discriminatory power was further increased by including the H2 locus in the MLSA strategy (14 sequence types). This analysis provided similar resolution than that obtained by 16S rDNA, though based in a much higher number of polymorphisms. It must be noted that Loc_12 and Loc_15 provided redundant information and may be excluded without loss in MLSA resolution. A robust phylogenetic tree was obtained by analysis of concatenated sequences (Fig. 2). Two lineages and five groups could be distinguished, which showed good correlation with geographic origins (Fig. 3). The isolate from Tajikistan (group 3) represents a distinct Asian clade; groups 1 and 5 are distributed in East Africa, whereas group 2 is present in Central Africa and group 4 in North Africa and Turkey. The presence of several groups in the Middle East may be explained by importation of animals from diverse origins for the religious feasts.

Number	Strain Designation	Country	Location	Year	Species	16S rDNA	H2	MLSA+H2	
1	97095 -Tigray	Ethiopia	Tigray	1995	Goat	-	Aa	1a	Lineage I
2	9277	Sudan	NK	<1992	Goat	-	Aa	1a	
3	99108 -P1 -C3	Eritrea	Barentu	1999	Goat	-	Aa	1a	
4	04012	Qatar	Doha	2004	Wild Goat	-	Aa	1a	
5	M74/93	Uganda	South East	1993	Sheep	IA	Aa	1b	
6	M79/93	Uganda	East	1993	Goat	IA	Aa	1b	
7	8789	Chad	Karal, Dandi	1987	Goat	IB	Ca	2a	
8	94156	Chad	N'Djamena	1994	Goat	-	Ca	2a	
9	05021	Sudan	Darfour, Nyala	<2005	NK	-	Ca	2a	
10	95043	Niger	Goure	1995	Goat	I	Cb	2b	
11	09018	Tajikistan	Rogun district	2009	Goat	-	D	3a	Lineage II
12	91106 -C550 -1	UAE	Dubai	1991	NK	IIA	D	3b	
13	Gabes	Tunisia	Gabes	1980	Goat	IIB2	B	4a	
14	LKD	Tunisia	Kebili, Douz	1980	Goat	IIB2	B	4a	
15	9081 -487P	Oman	NK	1990	Goat	II	B	4a	
16	07033	Turkey	Elaz ig	2007	Goat	-	B	4a	
17	Gabès/102p	Tunisia	Gabes	1980	Goat	IIB2a	B	4a	
18	7/2	Oman	NK	1988	Goat	-	B	4b	
19	97097 -Errer	Ethiopia	Errer	1997	Goat	-	Ac	5a	
20	89110 -C758 -C2	Sudan	NK	1981	Goat	IIB	A	5b	
21	97017 -Yatta	Kenya	Yatta	<1997	NK	-	A	5b	
22	F38	Kenya	NK	1976	Goat	IIB	Ab	5c	
23	94029 -C5	Oman	NK	1994	Goat	-	A	5d	
24	91039 -C3	Ethiopia	NK	1991	Goat	-	A	5e	
25	9231 -Abomsa	Ethiopia	Abomsa	1982	Goat	IIB1	A	5f	
26	92138 -chèvre1	Ethiopia	NK	1992	Goat	-	A	5f	

Table 1: List of strains analyzed showing corresponding 16S rDNA, H2 and MLSA sequence types

Material and Methods

The choice of loci for MLSA was done according to comparison of almost complete genome sequences of three Mccp strains (DNAStar, Lasergene SeqMan Pro V8). Twenty-two polymorphic loci were initially selected and their polymorphism was evaluated on six strains. This allowed the selection of 7 loci for the MLSA scheme. The analysis was then extended to a representative sample of strains (Tab. 1). Locus sequences were concatenated into a single sequence (Fig. 1) used for phylogenetic analysis (Darwin 5.0).

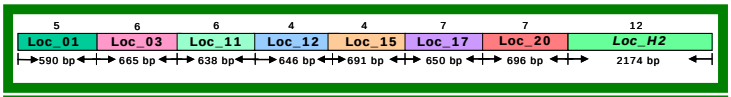

Fig. 1: Concatenation of locus sequences used for tree construction. Figures on top correspond to the number of polymorphisms for each locus.

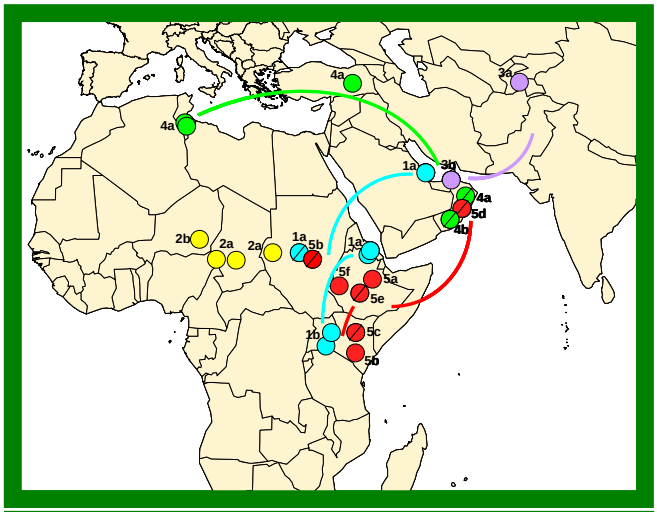

Fig. 3: Geographic origins of Mccp strains tested showing the distribution of MLSA groups. Colored lines represent links that may be explained by trade and other movements of animals. Note that when the isolation location was not known circles were barred and were positioned arbitrarily.

Conclusion

This new typing tool may be used for molecular epidemiology studies provided that representative numbers of strains (DNA) from affected regions are available. Still, our results strongly suggest that the recent outbreak of CCPP in Tajikistan is not due to introduction of Mccp in Asia but that it was most likely already present there.

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(3) **Yaya *et al.* (2008).** *Vet Res* **39**, 14

Centre de coopération internationale en recherche agronomique pour le développement
Département BIOS
Contrôle des maladies animales exotiques et émergentes
TA A15/G
34398 MONTPELLIER CEDEX 5, France
Tel: + 33 467 59 37 24
Contact: L. Manso-Silván
Lucia.manso-silvan@cirad.fr